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(54) **Human immunodeficiency virus GAG-encoded proteins.**

(57) The invention concerns nonfused recombinant proteins corresponding to the complete HIV gag and the complete HIV p17-9 subregion coding sequences, which are useful immunological components of diagnostics, therapeutics, and vaccines.

FIG. 2

Human Immunodeficiency Virus gag-encoded Proteins

FIELD OF THE INVENTION

The present invention concerns proteins derived by molecular cloning that are useful immunological components of diagnostics, therapeutics, and vaccines for infectious diseases. In particular, the invention relates to proteins encoded by the gag gene of human immunodeficiency virus, the etiologic agent of acquired immunodeficiency syndrome.

BACKGROUND

The human immunodeficiency virus (HIV, also LAV, HTLV-III, or ARV) is the primary etiologic agent of the acquired immune deficiency syndrome (AIDS) [Barre-Sinoussi et al., Science 220:868-871 (1983); Gallo et al., Science 224:500-503 (1984); Levy et al., Science 225:840-842 (1984)]. The underlying disease state involves a tropism of HIV for the T4 lymphocyte subset resulting in a selective depletion of the helper/inducer cells of the immune system, leaving the HIV-infected individual defenseless against a number of opportunistic infections. Approximately two million people in the United States and five million or more individuals world-wide may now be infected by HIV. The U.S. Public Health Service now estimates that the cost of caring for AIDS patients in 1991 will be between $8 billion to $16 billion per year. Thus, the development of diagnostics, therapeutics, and vaccines to HIV is the subject of intense medical research.

The nucleotide sequence of several independent viral isolates of HIV have been determined [Ratner et al., Nature 313:277-284 (1985); Muesing et al., Nature 313:250-258 (1985); Wain-Hobson et al., Cell 40:9-17 (1985); Sanchez-Pescador et al., Science 227:484-492 (1985)]. HIV is related to the lentivirus subgroup of retroviruses. Lentiviruses are associated with slowly developing, non-tumorigenic diseases in other animal systems. The major structural proteins of the HIV infectious particle are encoded by the viral gag, pol and env genes. In addition to the gag,pol, and env genes, the genome of HIV contains several other open reading frames designated sor,tat, art/trs and orf which encode additional viral proteins. These proteins are known to serve important regulatory functions during the HIV infectious cycle.

The gag gene products are involved in the structure of the genomic RNA-containing core and the assembly of the virus. The gag gene is termed "gag" for group-specific antigen, since in other retroviral systems the analogous gene encodes proteins that are cross-reactive with similar proteins of a related group of retroviruses. The HIV gag gene encodes a precursor of about 55,000 daltons (55 kDa) which is designated p55^{gag}. The gag coding sequence of HIV isolate BH10 (HIV$_{BH10}$) (Ratner et al., op. cit.) is contained within nucleotides 334 to 1869 and is comprised of 512 codons (Figure 1). The nucleotide numbering is according to Ratner et al. (op. cit.).

During the HIV infectious life cycle, the gag precursor p55^{gag} is proteolytically processed into species of approximately 24, 17, and 15 kDa. The gag proteins are designated p24^{gag}, p17^{gag}, and p15^{gag}. The mature gag proteins are derived from subregions of the p55^{gag} precursor in the order N-p17-p24-p15-C (N- and -C designate the amino and carboxyl terminus of the protein, respectively).

As is the case for the N-terminal gag-encoded proteins of other mammalian retroviruses [Henderson et al., Proc. Natl. Acad. Sci. 80:339-343 (1983); Rein et al. Proc. Natl. Acad. Sci. 83:7246-7250 (1986)], the N-terminal gag-encoded p17^{gag} is modified at its mature N-terminal glycine by myristylation. The N-terminal lipid myristic acid moiety of p17^{gag} presumably mediates the interaction of p17^{gag} with the lipid membrane of the virus particle. Gag-derived determinants are known to be exposed on the surface of cells infected by other mammalian retroviruses [Schiff-Maker and Rosenberg, Virology 154:286-301 (1986)]. Thus, HIV p17^{gag}-specific epitopes may be present on the surface of infectious HIV particles and HIV-infected cells. The p24^{gag} protein is the major virus capsid or core protein. The C-terminal gag-encoded p15^{gag} protein is highly basic and likely constitutes the core ribonucleoprotein and binds nonspecifically to many sites on the viral RNA.

Earlier reports describe the expression of subregions of HIV gag protein in heterologous expression systems such as in E. coli [Dowbenko et al., Proc. Natl. Acad. Sci. 82: 7748-7752 (1985); Chang et al., Science 228:93-99 (1985); Ghrayeb et al., DNA 5:93-99 (1986); Steimer et al., Virology 150: 283-290 (1986); Shoeman et al., Anal. Biochem. 161:370-379 (1987)] and in yeast [Kramer et al., Science 231:1580-1584 (1986)]. These recombinant gag-derived proteins produced in heterologous expression systems were shown to be useful as antigens for the detection of HIV gag-specific antibodies in human sera. No previous report has described the production using a het-

erologous expression sys'em, for example an E. coli plasmid expressio 'stem, of proteins corresponding to the full length, nonfused gag precursor p55⁹ᵃᵍ or to the mature gag subregion p17⁹ᵃᵍ. The present invention provides the full length nonfused HIV gag precursor p55⁹ᵃᵍ and the mature gag subregion p17⁹ᵃᵍ as produced using a heterologous expression system.

SUMMARY OF THE INVENTION

The invention provides proteins corresponding to the full length, nonfused HIV gag precursor p55⁹ᵃᵍ and the mature nonfused p17⁹ᵃᵍ subregion of p55⁹ᵃᵍ which are produced using molecular cloning methods and heterologous expression vector systems. Polypeptides corresponding to p55⁹ᵃᵍ and p17⁹ᵃᵍ of the HIV isolate BH10 (Ratner et al., op. cit.) were expressed in E. coli. Protein p55⁹ᵃᵍ is encoded by nucleotide number 334 to 1869 (nucleotide numbering according to Ratner et al., op. cit.) (see Figure 1). The p17⁹ᵃᵍ segment of p55⁹ᵃᵍ is encoded by nucleotides number 334 to 729. The HIV gag proteins are useful immunological components of reagents to identify individuals and blood products that have been exposed to HIV, of reagents that can be used to monitor and stage the progress of the disease in clinical therapeutic trials, therapeutic agents able to control the disease and of a vaccine that will protect Iduals who are exposed to HIV.

DETAILED DESCRIPTION OF THE INVENTION

Two recombinant proteins containing only amino acid sequences corresponding to the gag coding sequence of HIV have been created. These recombinant gag-derived polypeptides are highly immunoreactive with antibody sera from individuals infected with HIV. The term "peptide" is well known in the art and refers to a compound of two or more amino acids. One of the peptides of the invention corresponds to the complete and nonfused HIV gag of HIVBH10, p55⁹ᵃᵍ is encoded by nucleotides 334 to 1869 (nucleotide numbering according to Ratner et al., op. cit.). A second one of the invention corresponds to the N-terminal gag-encoded protein, p17⁹ᵃᵍ. In HIVBH10, p17⁹ᵃᵍ is encoded by nucleotides 334 to 729 (Ratner et al., op. cit.). The specific domains of HIV are known in the art.

The present E. coli expressed proteins corresponding to HIV p55⁹ᵃᵍ and p17⁹ᵃᵍ are referred to as GAG55 and GAG17, respectively. The structures of plasmid expression vectors designed for the expression of GAG55 and GAG17 in E. coli are detailed in Example 1.

As used herein, the term "expression vector" includes a DNA molecule which contains signals, recognized by a particular host biological cell, that direct and determine the expression of a particular gene sequence in the host cell. The vector DNA may, for example, contain information that determines the uptake of the vector DNA by the host cell, the integration and replication of the vector DNA, and the corresponding RNA biosynthesis and translation. Examples of expression vectors include recombinant viruses, plasmids and genes.

It is to be understood that the term "corresponding to" includes modifications of the specified amino acid sequences which do not adversely affect the antigenic characteristics of the peptide of the invention. For example, different isolates of HIV are known to differ to some extent in the predicted amino acid sequence of the gag-derived proteins (Ratner et al., op. cit.; Muesing et al., op. cit.; Wain-Hobson et al., op. cit.; Sanchez-Pescador, op. cit.). One skilled in the art could align the amino acid sequences of peptides from different sources to the schematic of Figure 1 or the nucleotide sequence of Ratner et al. (op. cit.) to identify the segments therein which correspond to the peptides defined herein.

HIV gag-derived proteins are useful immunological components of diagnostics, therapeutics, and vaccines for HIV-related disease, including acquired immune deficiency syndrome (AIDS). HIV-expressed gag proteins are known to be useful immunological antigens for the detection of HIV gag-specific antibodies [Petricciani, Ann. Int. Med. 103:726-729 (1985)]. The analysis of HIV-specific antibodies in human serum samples is extremely important in diagnosis of disease and for screening of human blood products. An enzyme-linked immunosorbent assay (ELISA) which uses HIV grown in tissue culture cells as antigen is now widely used for detecting sera positively immunoreactive to HIV (Petricciani, op. cit.). The ELISA derived from purified HIV particles grown in tissue culture is designated HIV-ELISA.

Since HIV infection leads to a series of clinical manifestations accompanied by variable antibody levels to different antigens, useful blood screening and diagnostic reagents should contain multiple antigens. Moreover, it is desirable to detect and quantitate antibody levels to specific antigenic components of HIV. Although the HIV-ELISA is a sensitive tool for the detection of HIV-specific antibodies in human serum samples, this test does not provide information as to which antigenic components of HIV are reactive with human antibodies.

The production of individu.. defi ... HIV-derived proteins, such as gag-enco... d p... ins, in E. coli provides antigen reagents . the .lective detection and quantitation of anti... ies : cognizing specific antigens, such as gac-: cod.. antigens. HIV gag proteins derived by n. .ec... cloning and expressed in heterologous ex: ress... systems, i.e., recombinant gag proteins, a .. : for the development of novel HIV diag... .. : k For example, the E. coli produced HIV (?.f .J5 ... GAG17 proteins can be efficiently u. ·ed a diagnostic ELISA for the detection ?.. .qu. ·itation of HIV. gag-specific antibodies that ... as. .iated with prior infection with HIV.

A heterologous expressic .v: ... such as that utilizing an E. coli plasmid r .... vector, provides a preferred alterna... t .ectious HIV grown in tissue culture as .. ur of antigen for ELISA blood screening a... ci.. .stic reagents. Importantly, the E. coli pla... d e: .ssion system is free of infectious HIV. M... .er, / gag-derived proteins GAG55 and GAG.. .an L.. expressed at high levels in E. coli using t... .a.. .riate vectors, thereby facilitating the purif .... these useful polypeptides. Thus, recom... ... can be produced in large quantities .. .c he biohazard and containment problems . with the production of gag proteins fro... ... ; HIV virions.

The recombinant gac ...: f the present invention are preferred to .. :.. described recombinant gag proteins. The .: ·· -inant product GAG55 corresponds to ·.e .. .h, non-fused product of the complet.. .g sequence. Previously described re: .. n .ag proteins only contain subregions c... .. .xample, in a recombinant gag protein ... .y Kramer et al. (op. cit.) and Shoema: .. .. cit.), amino acids number 2 to 12 of .. .. .leted. Other reported recombinant gac . t . reported have larger segments of the ·.5 .. .nce deleted (Dowbenko et al., op. c:.. .. al., op. cit.; Ghrayeb et al., op. cit.; St.· .. op.cit.). More gag-specific antigenic de.. re represented by the product of the .n .. .ll length gag coding sequence than a.. .. .d by incomplete subregions of gag. .. roduct of the full length gag coding so .. .h as GAG55, is expected to be a more .. .agent for the detection of gag-specific .. .. .ian are antigens comprised of only s .t. gag. .

In particular, GAG55 .. 7 contain the region of gag corresp. he complete p17gag. This complete regi... .. s not present in other reported recon... .. proteins. As discussed previously and .. ..17gag region of gag contains antigenic .. that may be presented on the 'surfac. .. HIV-infected cells, and may be crit. .. .tective host immune response to HIV.

Another advantage of the peptides of the invention is that the recombinant gag-derived proteins are not fused to other, non-HIV, or non-HIV gag sequences. The inclusion of additional non-HIV sequences, such as E. coli-derived sequences, fused to the HIV gag coding sequence is not desirable since these sequences could lead to frequent false-positive (i.e., non-HIV) antibody reactivity. Thus, the HIV gag sequences have been expressed in E. coli in the absence of fused bacterial or other non-HIV protein sequences. It is also desirable to express nonfused authentic gag proteins, rather than fusion proteins, in order to facilitate the correct folding and native structure of the product. The correctly folded structure of the product may be critical to its effectiveness as a diagnostic, therapeutic, or vaccine reagent. Previously reported recombinant HIV gag-derived proteins are fusion polypeptides containing non-HIV sequences.

Although most current strategies for the development of an AIDS vaccine are focusing on the HIV env gene, the gag antigens may well be an important component of a protective vaccine against HIV. Indeed, recent reports suggest that gag p17gag antigenic determinants are accessible to the neutralizing effects of specific antibodies [Sarin et al., Science 232: 1135-1137 (1986)]. As discussed previously, p17gag determinants may be exposed on the envelope of the virus itself, since the gag p17gag protein is thought to be associated with the virus lipid membrane.

AIDS patients are less likely to have antibodies to gag-encoded proteins compared to individuals with milder forms of the disease [Steimer et al., Virology 150: 283-290 (1986)]. Thus, there is at least correlative evidence that the gag-specific host immune response may be critical for the effective control of HIV by an HIV-exposed individual.

Due to the heterogeneity in the sequence of env from various HIV isolates [Ratner et al., Nature 313:277-284 (1985); Wain-Hobson et al., Cell 40:9-17 (1985); Muesing et al., Nature 313:250-258 (1985); Sanchez-Pescador et al., Science 227:484-492 (1985)], it is possible that a vaccine containing env from a single HIV isolate may not have a broadly neutralizing effect on other HIV species. The degree of heterogeneity in the gag gene product among different HIV isolates is far less than that found for env [Ratner et al., Nature 313:277-284 (1985); Muesing et al., Nature 313:250-258 (1985); Wain-Hobson et al., Cell 40:9-17 (1985); Sanchez-Pescador et al., Science 227:484-492 (1985)]. Thus, HIV gag gene products may be an important component of a vaccine that is broadly protective against many isolates of HIV.

Since the peptides of the invention are produced by recombinant DNA technology in the ab-

sence of infectious H /, ... y provide several advantages over more : ditior I vaccine approaches using killed or atte·uate 1 : preparations. Recombinant peptides .·e s.. . repare and administer and no genetic ·nfor..1a is introduced by vaccination. In additi·n re:: inant peptides are more readily produce.i and · ..fied than the viral-expressed peptides ·sol·t· 1 frcm virus-infected cells.

In one embodim· , ·h·)eotide of the invention is used in a di .jno · · · used for detecting antibodies to HIV in .· bi· sample. The peptide can be employ· · in · :ess for detecting antibodies to HIV cr ¬pri· · ntacting a biological sample with th·· pe·t: ind detecting immunoreactivity. The p·)ti ·· :an ·lso be employed as a component in .· v: · ·ine protective against HIV. The vaccine cc· ri·· ·n ·ffectively protective amount of the p· . .c· .

E. coli strain KA·29t ·rned with plasmid pGAG55, encoding ·)tei.. L 55 and described in Example 1, has t ·:n r .· ·d with the American Type Culture ..)lle: · ATCC), Rockville, Maryland, and bears d· :t accession number 67378. This deposit is av.·)le t·· the public upon the grant of a patent ·· i · ·ss!·nee. However, it should be understoc. .. · .vailability of a deposit does not cons· .t· ·· · ·· ·e to practice the subject matter in de)·.· iient rights granted by governmental :io.

Materials and Method:

Unless otherwis· ·· i, · ·rts and percentages are by weight ·· ·or ·re Celsius.

Plasmid Constructio·

Plasmid constru·· :. v.re ·arried out using standard methodolog· .. . crit .d by Maniatis et al., Molecular Cloni·r .·i ·· ·v Plasmid, Cold Spring Harbor Labc ·or ·. · ·York (1982), the teaching of which is ··)orated by reference. Enzymes and · · · · .. .s used for plasmid constructions .· : from Bethesda Research Laboratori· , ·... ·urg, MD or New England Biolabs, Be· ·· .. · · .thods for digesting, identifying, recc· · · .·d ·urifying the various nucleotide seq·· · · · in the invention are known to those ·· .. . ·rt as are methods for ligating the . · ·· vectors, transforming host micro .· ·· ·1s, cloning, and recovering product· .· · Accordingly, the methods will only ·e c· by reference to specific embodime·: · ·ention set forth hereinafter.

E. coli Strains, Plasmid Expression Vectors, and Induction of Expression

Several types of E. coli plasmid expression vectors were used for the production of HIV gag-derived proteins in E. coli. In the vectors used, expression of the desired product is controlled transcriptionally using either the operator and promoter of the E. coli tryptophan operon [Ivanoff et al., Proc. Natl. Acad. Sci. 83:5392-5396 (1986)] or the phage lambda P_L promoter [Rosenberg et al., Methods Enzymol. 101:123-138 (1983)]. We have also used the E. coli lactose operon promoter to direct expression of HIV gag-derived proteins in E. coli.

Plasmid vectors utilizing the phage lambda promoter were derived from plasmid pBF106. The E. coli plasmid expression vector pBF106 was constructed as follows. The small HpaI to BamHI fragment of plasmid pKC30 [Rosenberg et al., Methods Enzymol. 101:123-138 (1983); Rao, Gene 31:247-150 (1984)] (obtained from K. Abremski, Du Pont Experimental Station, Wilmington, DE) was replaced with the following oligonucleotide linker:

5´- AAC GAA TCC GAA GTG TAA GCC ATG -3´
3´- TTG CTT AGG CTT CAC ATT CGG TAC CTAG -5´

The inserted sequence provides signals for efficient translation initiation and a unique NcoI restriction endonucleose site. Since the translation start ATG forms part of the NcoI site, coding sequences can easily be fused in-frame directly to the ATG and translation initiation signal.

Expression from the P_L-containing plasmids is controlled transcriptionally using a lysogenic host that provides lambda cI repressor. Expression is induced by inactivating cI using nalidixic acid [Mott et al., Proc. Natl. Acad. Sci. 82:88-92 (1985)] or by temperature-shift and using a temperature sensitive repressor such as cI857 [Rosenberg et al., Methods Enzymol. 101:123-138 (1983)]. Temperature-shift inductions were carried out using host strain KA1298 (obtained from K. Abremski, Du Pont Experimental Station, Wilmington, DE), according to the procedure of Young et al., Proc. Natl. Acad. Sci., 80:6105-6109 (1983). KA1298 is a defective lambda lysogenic containing cI857. Nalidixic acid inductions were carried out using the defective lysogenic host DC550 (obtained from D. Court, NCI, Frederick, MD), according to Mott et al., Proc. Natl. Acad. Sci. 82:88-92 (1985).

E. coli plasmid expression vectors containing the tryptophan promoter were derived from pKGP36.trp (Ivanoff et al., op. cit). E. coli host strains MM294 and HB101, DH5 (F¯ recA1, endA1, gyrA96, hsdR17, supE44), DH5Δ (lac), SC122, CAG456(htpR165), CAG629(lon-htpR165), and SG4119 (Δlon) were used [Maniatis et al., Molecu-

lar Cloning: A Laboratory M.. .al, Cold Spring Harbor Laboratory, NY (1982); .. er et Proc. Natl. Acad. Sci. 81:6779-6783]. _. `...` .m the tryptophan promoter was c...d o described (Ivanoff et al., op. cit.).

Serum Samples and Serology

Human sera were coll.. . i at .. .d Bank of Delaware blood donor cen' .s `...` . 86 or were obtained from T. Mathews Duke .. -ity. Serum was stored at -70°C befc .e testii d repeated freezing and thawing was avoic preliminary classification of all sera was ma. .sed on .repeated tests with an HIV-ELI.`A (Du H.nt, Wilmington, DE) blood screening ki . on the results of HIV immunoblot testing carriec .. t by commercial laboratory (Biotech Rese? .n a.. ..ies, Rockville, MD), and on testing .._r? io vity to env using a recombinant antig..n (E.. .ed ELISA (described below). Proce.ur? .. 3-1/2 hour assay described in the Du Pc..... - III ELISA blood screening kit, was us.. .. to .... era. Further immunoblot analysis was ...ried out using commercially available HIV ant .-cont..r.ng nitrocellulose strips (Du Pont-Bio.. .. ..e.. . Laboratories).

Immunoblot Analysis

Standard methods for .. ...cryla. ...de gel electrophoresis (SDS-PAGE) [L.. ..nli. ... ..re 227:680-685 (1970)] and electroph ... of ...oteins to nitrocellulose [Towbin .. a ...tl. Acad. Sci. 27:4350-4354 (1979).. .. .de preparations of GAG55 (30 ng .....ta .rotein per lane were typically used ror .. .. t analysis. Following electrophoretic tr.....i, ...rocellulose strip were preincubated 3.. ... room temperature In Blotto buffer .. ..on-.at milk solids, 0.05% Tween 20, ...at serum, phosphate buffered .. .... s were then incubated with a d...... .:50 in Blotto buffer) of sample ....v for 1 hour to overnight at 4°C. .. 'os.. strips were then washed exhaus.... .. s..ha.e buffered saline containing 0.. .. -20. Human immunoglobulins were c .. ..all.. using biotinylated anti-human ..li.., and avidinD-horseradish pero .. ... .Vector Laboratories, Burlingame C.. . ...tion of sera for competition exp... ... ..cuba- tion of 10 to 20 µl of ser. ..i. ..o..nes of E. coli extract (containi..n) .. .. i b.cterial protein) for 2 hours at 25° (.c - centrifugation to remove insoluble m.. .. .

Protein Purification

E. coli-expressed GAG55 was purified by immunoaffinity chromatography. Induced E. coli were collected by centrifugation and the cell pellet was stored at -70°C. The induced E. coli cell pellet was suspended in PBS and the cells were lysed using a French press. The lysate was centrifuged (27000 x g) and the supernatant applied to an immunoaffinity column containing p17gag mouse monoclonal-specific antibody BT2 (Biotech Research Laboratory, Rockville, MD) covalently attached to Reacti-gel (Pierce Chemical, Rockford, IL). The immunoaffinity column was prepared as specified by the supplier and washed and equilibrated with phosphate buffered saline (PBS). The E. coli cell lysate was loaded onto the column and the column was extensively washed with PBS. Fractions containing gag protein were eluted from the column using 0.5 N acetic acid and were then pH neutralized, lyophilized, and dissolved in PBS containing 0.1% SDS.

Alternatively, GAG55 was purified from a French press lysate of E. coli by cation exchange chromatography. Soluble proteins were dialyzed against 0.05 M phosphate buffer (pH 7.0), applied to a Pharmacia Mono S column (Pharmacia, Piscataway, NJ) and eluted with a NaCl gradient. The purity of the antigen was approximately 90% purified.

The peptide ENV9 was purified from E. coli containing the vector pENV9, as discussed in assignee's copending application, serial number 010056, filed Feb. 2, 1987. The predicted protein product of pENV9, designated ENV9, contains 340 amino acids which correspond to 54 amino acids from the N-terminal of the poliovirus sequence of pEXC [Ivanoff et. al., op. cit.], 46 amino acids of the C-terminal of the HIV env gp120 domain and 340 amino acids of the N-terminal of the env gp41 domain. Enzyme Linked Immunosorbant Assay (ELISA)

Purified GAG55 antigen in 60 mM Na_2CO_3 pH 9.6 buffer containing 0.0005% SDS was applied to Immulon II microtiter plates at 100 ng/well at 4°C for about 18 hours. Purified ENV9 antigen was similarly applied in 60 mM carbonate pH 9.6 buffer containing 0.00006% SDS at a concentration of 20 ng/well. ELISA procedures were carried out as specified for the commercial HIV-ELISA kit (Du Pont, Wilmington, DE). The antigen-coated plates were washed with PBS + 0.05% Tween 20 (PBS-T). Plate washings were performed using 3 cycles on a Titertek Microplate Washer 120°followed by rotating the plate and washing again. The plates were incubated with PBS-T for 1 hour at 37° and were then washed 3 times with PBS-T and stored dry at 4° until they were used.

The plates were incubated with patient sera at

a 1:20 to 1:100 dilutic: n d
bovine serum albumir .¾
mal goat serum (NGS . .¾
thimerosal) in the mic   ɜr w
2 hours at 25˚. The ˜ .˙ɜs v
PBS-T, exposed to gc ᴺti-¹
to   alkaline   phosr   ta_
munoResearch, West ⌐ -ɛ,
and washed with PBS-⁷ ͺ\ɛ
reaction was develop⸗c ˉ ˍ
plier, by exposure ˙ /C
phosphate in 100 µl c ..ɜth⸗
pH9.8) with magnesi ⁻ ch¹
dium azide at a for 3 ⁱnuᵗ
addition of sodium hyc⸗ ¹c¹
1 N. The plates were ⁴ ⸗
MCC microtiter plate re ˙ ˉ
nm.

͵r⸗S-T + 5%
ᴵnactivated nor-
.ͺ ⸗ꜟde, 0.05%
⸗ .ͺ⸗ubated for
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⁻ᴺcᴺtration of
⸗ ᴺ Multiskan
ᴺcᴺth of 405

FIGURE 4: Immunoblot reactivity of E. coli-expressed GAG55-derived proteins with human AIDS patient serum antibodies. Total E. coli lysates were analyzed. Lane 1: DC550/pGAG55, non-induced; lane 2: DC550/pGAG55 nalidixic acid-induced; lane 3: DC550/pBF106, nalidixic acid-induced.

FIGURE 5: Binding of gag-specific antibodies in human HIV-seropositive sera by E. coli-expressed GAG55, as analyzed by competition binding and immunoblot. Prior to immunoblot of HIV antigens (Biotech Research Labs, Rockville, MD), human HIV-seropositive sera were preincubated with cell extracts prepared from induced E. coli, as indicated. Lane 9: DC550/pBF106, 20 µl extract; lane 10: DC550/pGAG55, 5 µl extract; lane 11: DC550/pGAG55, 20 µl extract. As shown, E. coli-expressed GAG55 protein effectively and selectively binds to all of the immunoblot-detectable gag-specific antibodies in this AIDS patient serum. Similar results have been obtained for several other HIV-seropositive sera. Thus, essentially all of the HIV gag-derived determinants and epitopes recognized by human antibodies are presented by E. coli-expressed GAG55. The figure also illustrates the identification of human serum samples containing HIV gag-reactive, HIV env- and pol-nonreactive antibodies. Human sera were analyzed by immunoblot of HIV antigens. In lanes 2 to 8, the specificity of the immunoblot reactivity for gag antigen was confirmed by competition with E. coli-expressed GAG55 protein (not shown).

FIGURE 6: Frequency distribution of GAG55-ELISA reactivity in three sample populations. Histograms show the fractions of samples in the absorbance ranges indicated on the horizontal axis. Absorbance ratio is ratio of the sample absorbance to that of a reference HIV positive control serum. Samples with absorbance ratios greater than 0.5 are grouped together for clarity. Upper panel: 69 normal HIV-ELISA-nonreactive blood donors. Middle panel: 57 representative HIV-ELISA reactive env-nonreactive sera from a blood donor population. Lower panel: 31 HIV positive sera: 26/31 of these sera are from patients with clinically diagnosed AIDS or AIDS related complex. Statistics for the populations were: normal sera, mean absorbance ratio = 0.00, s.d. = 0.03; HIV-reactive blood donors, mean = 0.13, s.d. = 0.11; HIV positives, mean = 0.37, s.d. = 0.43.

FIGURE 7: Comparison of apparent HIV gag-specific and env-specific antibody levels in human sera analyzed by ELISA. Antigens ENV9 and GAG55 were purified following expression in E. coli. Symbols: open triangles, HIV-seronegative; open

BRIEF DESCF ᴵOᴺ   ᴺ˙ ᴸ ᴱS

FIGURE 1: Nucᴵ ᴺe
region of HIVBH10 (Ratᴵ ⸗
of the gag p55ᵍᵃᵍ codi. eᴸ
an open bracket and ᴸw;
p55ᵍᵃᵍ coding sequer . ᴺᴸ ⸗
bracket. The junctio˙ .ᴺ
p24ᵍᵃᵍ polypeptides, t .rᴵ⸗
cessing of p55ᵍᵃᵍ, is ᴵcᴸ
comprised by gag aᴵr ᴸ
p17ᵍᵃᵍ is comprised b.
to 132.

c⸗ the gag
ᴺᴺ. The start
ᴸᴸ ͺᴸicated by
ͺᴺᴸ of the gag
ᴸ ᴸᴸy a closed
͵17ᵍᵃᵍ and
⸗lytic pro-
. p55ᵍᵃᵍ is
ᴵ ᴸ to 512;
ᴺumber 1

FIGURE 2: Scᴵ˙ ᴵᴺ
coli plasmid vector dɛ ᴸᴸ ⸗
non-fused HIV gag pr⸗ ᴺᴺ
mid pGAG55 (pgag5ᴸ ᴵ
from pBR322 (thin lin ᴺ⸗
shatched line), and r ⸗
well as a chemically ᴸᴸ
(thick closed line). In ᴸᴸ
gag coding sequenc⸗ ᴼr
lambda PL promoter ᴿᴸ
nals derived from a ᴸ ᴺᴵᴸ
sequence. As indicatᴸ ⸗
preceding the gag Aᴵ ᴵᴺ
have been changed ᴵr ᴸ⸗
erating a unique Ncol

⸗⸗ ᴺtion of E.
. ᴺ . ᴵull length,
ᴸ⸗ᵍᵃᵍ. Plas-
ᴺ⸗ derived
ᴺhick cros-
. line), as
ᴺ segment
ᴸᴼn of the
ͺᴺ the phage
ᴵ ᴵ⸗ation sig-
⸗ ized DNA
ᴵes 5' and
ᴺ HIVBH10
⸗⸗by gen-
ͺᴸase site.

FIGURE 3: Exᴸ
The accumulation of 5ᴸ
lyzed by SDS-PAGE
Coomassie staining. Lᴸ ᴵ ᴸr
ing pBF106 (DC550/ᴸ˙ ɛᴸ
acid. Lane 2: DC55C
3: DC550/pGAG55, inᴸ

ᴺ E. coli.
. ᴸ ᴸ. ᴸas ana-
ᴸᴸ sates and
⸗ ᴸ contain-
ᴺ nalidixic
ed. Lane
ᴺcid. The

circles, non-AIDS, HIV-serop?s .ve;   uares,
AIDS patients HIV-seroposit.   App'   / gag-
specific antibody levels are ., . 'c   .ced in
AIDS patient sera compar. ' ˍ ·   ) HIV-
seropositive sera. This app :   'e iv‹   :rease
in gag-specific antibody le   ˼ ˍ . ﹦'   since
env-specific antibody level˼   ɘ n' ˻ s₁   ly af-
fected.

EXAMPI

The invention is furthe ‥ ¬ri' ꓸ   ꟷ⁹ fol-
lowing Examples, wherein   ꓰart ꜛ.   ꞋIcent-
ages are by weight and degꞌꜟ   are ꓲel˼

EXAMPI

Plasmid Constructions

HIV sequences were ob'ꜛ' .d fr﹍   ɔroviral
clone λ-BH10 (Ratner et   ꓹꓸ.   ꞊ HIV-
containing SacI fragment oꟷ :   .   ꓷs iso-
lated and treated with DNAꜛ   ꞉ꞈ   ions of
limited digestion to generꜛ.   ꓲꓵꞈ ꓵ   ꞊ frag-
ments. The randomly cut H' ꜛꓸ 'A   ꓸ˼ were
blunt ended by Klenow reaꞈ ꓲ꜏ ꓹ liꞇ ꜛu   ꓸlindlll
linker, cut with HindIII, and ꓲꓸꓲ ꓲed ꓸto   unique
HindIII site of plasmid pTꓳꓴꞈ   Pꓲꞈ   TORF2
is derived from pBR322 (ꓲ ꓷ ꟷ   ⁷); se-
quences inserted at the   'ꓲe are
transcribed under the contrꞇ ꓸ ꓹ   ꓰ. coli
tryptophan operator and p ꓸ 'ꓸꞏr   et al.,
op. cit.). The resulting plaꞏ ꓸ ꓸ꜏꜏ ꓸ꜏   ꜏ were
used to transform E. coli   ꓲꞏa   ꜛꞈ con-
taining HIV-derived sequer ꜛ꜏ wꓸ ꓲꞈ   'ed by
colony hybridization using ꓲꓸ tꞍꜛ ꓸ   ꓸIV𝐁𝐇10
proviral DNA as a probe ꓸ ꜛꞇ   ꜏smids
containing HIV-derived seꞈ   ꜏nated
pGAG3, pGAG4, PR2. pG ꜛꓸꜟ   ꓸ PR2
were shown by DNA sequ꜏   ꓲꓲ   ꜏ɔntain
HIV-derived DNA inser ꜏ꓷꞇ ꜟ   ꞇ to
nucleotides 227 to 1010, ꓷ   ꓸ   ꜟ29 to
2037, respectively. HIV nuꜛ ꓲꓲꓸ ꓹꓸꟷ   ꓲbered
according to Ratner et al. ꓹ   ꞇ   ꓸIV𝐁𝐇10
gag coding sequence ꓸ ꓹꓲ   ꓹvithin
nucleotides 334 to 1869 (GAG3
and pGAG4 contain the 5' ꜛꓲ ˻   ɔding
sequence and the HIV-dꜛ   2 ex-
tends beyond the 3' end   ꞇ se-
quence.

Plasmids pGAG9 and   ꞏecify
the expression in E. coli un.   yꟷ   ꟷoter

control of the complete HIV𝐁𝐇10 gag coding se-
quence, were derived as described below. pGAG9
was derived by ligation of the following DNA frag-
ments: 1) the PstI to PstI tryptophan promoter-
containing fragment of pGAG4, 2) the PstI to PstI
pBR322 origin-containing fragment from pR2.
pGAG10 was similarly derived by ligation of the
following DNA fragments: 1) the Pst to PstI trypto-
phan promoter-containing fragment of pGAG3, 2)
the PstI to PstI pBR322 origin-containing fragment
of pR2. In this way the PstI site within the gag
coding sequence was used to combine the 5' of
the gag-coding sequence from pGAG3 and pGAG4
with the 3' of the gag coding sequence of pR2, to
generate pGAG10 and pGAG9, respectively.
pGAG10 places HIV sequence from nucleotides
227 to 2037 under transcriptional control of the
tryptophan promoter. pGAG9 places HIV sequence
from nucleotides 273 to 2037 under tryptophan
promoter control (see Figure 1). E. coli translational
control signals for translation initiation at the gag
ATG at nucleotide 334 are provided by the HIV
sequences immediately 5' of the gag ATG. These
translation initiation signals were predicted to be
functional in E. coli on inspection of the HIV se-
quences.

Plasmid pGAG55 (Figure 2) is constructed by
ligation of the following three DNA segments: 1) the
large P𝐋-containing BamHI to NcoI fragment from
pBF106; 2) the HIV gag-containing ClaI to BamHI
DNA segment from pGAG9 or pGAG10; 3) a NcoI
to ClaI oligonucleotide of the following sequence:
5'- C ATG GGT GCT AGA GCG TCA GTA TTA
AGC GGG GGA GAA TTA GAT-3' 3'- CCA CGC
TCT CGC AGT CAT AAT TCG CCC CCT CTT AAT
CTA GC-5' The NcoI to ClaI oligonucleotide con-
tains sequence information coding for the first 14
N-terminal amino acids of gag. Plasmid pGAG55
thereby contains the complete HIV𝐁𝐇10 gag coding
sequence fused to the synthetic E. coli translation
initiation signal in plasmid pBF106, as described
previously (see p. 14). Plasmid pGAG55 is also
referred to as pBF128. A unique NcoI site has been
engineered at the translation start ATG of gag
without altering the gag coding sequence (see Fig-
ure 2). The complete gag coding sequence can
now be conveniently moved from GAG55 as a NcoI
to BamHI DNA fragment cassette to other expres-
sion vectors. For example, the gag coding se-
quence can be precisely fused to signals directing
the expression of the full length gag precursor in
mammalian cells. Importantly, HIV sequences 5' to
the gag coding sequence have been eliminated in
the NcoI to BamHI DNA cassette. HIV sequences
immediately 5' to the gag coding sequence, includ-
ing a splice donor signal, are very likely to limit
expression of gag in mammalian cells.

Plasmid GAG17, which specifies the expres-

sion in E. coli of the .. ture, .   .d p17^{gag}
protein, was constructed t lig   following
three DNA sequences: i   e   .rived, ap-
proximately 2962 base p. S   .   ONA frag-
ment from pGAG55 (see Figu .):   a PstI to
PvuII fragment from pGAG55 (of .xi.   .tely 3068
base pair) containir pBF   les   ed DNA,
lambda-derived DNA, a he ſ   c.   f HIV gag
(see Figure 2); 3) a chei. lly sy   -   d PvuII to
SalI DNA segment of the 'ic   nce (only
1 DNA strand is shown):
5'- CT GAC ACA GGA   : /   CAG GTC
AGC CAA AAT TAC T /. λ -   's   ull to SalI
fragment extends the gar cor ic _   nce from
the PvuII site at nucleoi Je   Fi   e 1) and
introduces of TAA stor don   -   Tyr codon
(TAC) at the p17^{gag}-p2. iur . = e 1). The
3' G residue of the indica d   s the first
nucleotide of a SalI sitr In   a coding
sequence correspondin   )r   cleotides
334 to 729 of HIV$_{BH10}$ i ir ; ; ed under
transcriptional and trans atior   derived
from pBF106. pBF106 is c cr r re   usly.

EXAM E

Reactivity of E. coli-e .   e   ,   with HIV-
Specific Antibodies

As shown in Figure ϡ ͻ   ,   n can be
efficiently expressed a: i. ,   n   coli, using
the appropriate plasmid re .   r system.
Analysis of total E. coli eins   C   AGE and
Coomassie blue stainii., _ic   ᴕ   AG55 ac-
cumulates to a level c rc   ᴕ of total
E. coli protein. This r g iev   ᴇ   ession of
GAG55 facilitates pu atio.   protein.
GAG17 similarly accu n tᴖ-   vel in E.
coli.
Monoclonal antib-   -   HIV-ex-
pressed p17^{gag} (BT2, f :h .   abs) and
p24^{gag} (BT3, Biotech F є rch   e found to
react specifically with (/ 5 ᴝ   en tested
in immunoblot and E   a   :   methods
described previously. - add z bbit anti-
serum specific for hi i pᴖ   p24^{gag} -
(obtained from D. Rᴖ . .   t   erimental
Station) was immunore.   ᴖ   r   xpressed
GAG55. Furthermore,   .o   GAG55
was shown to be imi rear   .   any hu-
man AIDS patient ser.   ᴖ r   iot assay
(for example, see Fig   .   coli-ex-
pressed GAG17 is im ᴝ ie r   :   p17^{gag}-

specific monoclonal antibody BT2 and many hu-
man AIDS patient sera. These results confirm that
viral antigenic sites reside within E. coli-expressed
GAG55 and GAG17.

EXAMPLE 3

Use of E. coli-expressed GAG55 as an Immunogen

Another advantage of the antigenic structure of
E. coli-expressed GAG55 is the ability of the pep-
tide to illicit HIV-specific antibodies in animals. Pu-
rified GAG55 was injected into rabbits and goats
and the resulting antisera were assayed by im-
munoblot and ELISA, according to the methods
described previously. Goat and rabbit antisera to E.
coli-expressed GAG55 reacted with the expected
HIV-expressed gag-derived proteins p55^{gag}, p24^{gag},
and p17^{gag} on viral immunoblot. Antisera to GAG55
also reacted with an HIV-ELISA (Du Pont).
The titer of the antisera to E. coli-expressed
GAG55 was found to be equal to or greater than
antisera to gag proteins purified from HIV in spe-
cifically binding to the HIV-expressed gag proteins.
This result demonstrates that E. coli-expressed
GAG55 contains the major native viral epitopes and
that antibodies to GAG55 are sensitive reagents for
detecting viral proteins.

EXAMPLE 4

Detection of Antibodies to HIV gag in Human Sera

The E. coli-expressed gag-derived protein
GAG55 is comprised of protein sequences cover-
ing the complete gag open reading frame and
should, therefore, be very similar antigenicaly to
P55^{gag} expressed by HIV in infected mammalian
cells. In order to compare the ability of GAG55 and
HIV-expressed gag antigens to be recognized by
human antibodies in HIV-seropositive sera, experi-
ments involving competition between recombinant
and viral gag antigens for reaction with serum
antibodies were carried out. Using a competition
immunoblot technique, six HIV-seropositive human
sera were tested for reactivity with GAG55 antigen
as described below. Sera were pre-absorbed with
GAG55-containing E. coli extract and, in parallel,
with a control E. coli extract. These absorptions
were followed by analysis of the sera using HIV

antigen strips (Biotech Resea , Labs Wi.. 1 each
HIV positive serum tested, :   mbinar.t gac poly-
protein was found to substar _ y, if nct coi)lete-
ly, out-compete reactivity .. II the gag- :iated
immunoblot bands (see Figu a 5,. The spec .ity of
this competition effect for c-g ·.as sh· v   · the
lack of competition of env or r)i bands   the
lack of effect of control E. c.· .tracts n / HIV
immunoblot band. The pres-' :oults ·m istrate
that most antibodies in HIV-   ·ositi· ser reac-
ting with HIV gag immunobl   nds also re t with
GAG55 antigen. Thus, E. cc   ·ressed G i5 is
clearly a useful reagent fo· tl · detec·ior · HIV
gag-specific antibodies and. the ·fore, ·-r   b· a
useful component of HIV .· .nostic ar .od
screening procedures. The: ·condu .s , in-
volve, for example, immunoh·   ·· ELIS .m ·ods.

Purification of gag-specific   ·>· ie· in ·man
HIV-seropositive serum by .. .y c.· nate raphy
using E. coli-expressed GAG

E. coli-expressed GAG   ·as demo· ı.ted
using immunoblot assays t. o· .i to e·se· ..· all
of the gag-specific antibodi· · .many · _ :IV-
seropositive sera (Example ·. ·ure 5 T ·re,
we prepared an affinity col r · r h· pr· ·tive
isolation of HIV gag-spec·   ... ·s. .·355·
was partially purified as   ribu.. .ab. and
covalently coupled to a Re· ·el su..port iorce
Chemical, Rockford, IL) a: ·ifi.d ·by t ·up-
plier. Human serum was lc to a · ·lur on-
taining the GAG55 affinity   · ·. t·. · ·as
washed extensively using   tl · .a· ·ed
human antibodies were el·· · ·v ... · ·he
eluted fractions were pH · ! ·m· .lot
analysis of HIV antigens (b· ·r ··-· L bs,
Rockville, MD) using the · 55-r·· ·ied ·iman
antibodies revealed select· ·m·unc·rear ·· of
these antibodies with gag· .i ·n·i sr · i ·se
results demonstrate the uti· · b· c. ·· .sed
gag protein for the prep... is· · . ·g-
specific antibodies.

HIV gag-specific antib .. · · b· · ı re-
search reagents for the id. · ·· · · . ·ng
of gag-specific epitopes an· ·· r .. ı·· · ·ıost
immune response to HIV. · ·ar·· · it ·· be
possible to identify gag-sp· . ·· · ·an
neutralize or block HIV inf· _ ·· _ · of
such HIV- neutralizing ar i ·. ·tic
reagents will depend on · ·ı· ..· ıor
their purification. As disc ·.. ! ·v c ·ull

length gag protein, such as E. coli-expressed
GAG55, that represents essentially all of the gag-
specific epitopes may be the preferred affinity
reagent for the identification and purification of
gag-specific antibodies. Subfragments of gag pro-
tein, as previously disclosed by others, can only
represent a subset of the total set of gag-specific
epitopes.

EXAMPLE 6

Identification of HIV gag-reactive, env- and pol-
nonreactive Sera

ELISAs utilizing as antigen purified HIV grown
in tissue culture (HIV-ELISA) have proved valuable
for screening blood for evidence of exposure to
HIV. Many times sera will repeatedly test positive
on HIV-ELISA but will not show clear evidence of
HIV exposure when subsequently examined by
HIV-immunoblot. During screening of blood donors,
we and others [Courouce et al., Lancet II:921-922
(1986); Biberfeld et al., Lancet II:289-298 (1986)]
often detect reactivity in HIV immunoblots at posi-
tions of gag antigens p15, p17, p24, or p55 un-
accompanied by detectable immunoreactivity with
env. Examples of such HIV gag-reactive, env-non-
reactive sera are shown in Figure 5. The specificity
of these human antibodies for HIV gag was dem-
onstrated by competition binding with GAG55.
Thus, preincubation of the sera with GAG55 spe-
cifically blocked binding of the antibody to the gag
proteins on the HIV-immunoblot.

More evidence demonstrating the occurence of
anti-gag antibodies and quantitative data on their
relative levels in different populations was obtained
using purified recombinant antigens in ELISA for-
mat as shown in Figure 6. The HIV-ELISA-reactive
blood donors are distinct from the normal group in
having elevated gag ELISA titres, as does the HIV-
positive group. In addition, the GAG55-ELISA val-
ues for the HIV-ELISA-reactive blood donors are in
the same range as several of the HIV-positive sera.
Analysis of these samples with the ENV9-ELISA
revealed that the absorbances of the HIV-ELISA
reactive donor group were distinctly lower than the
HIV-positive group and similar to those of normal
sera.

For a high percentage of the more than 200
HIV-ELISA positive sera that we have examined,
there is unequivocal evidence of antibodies that are
immunoreactive with HIV gag antigens, unaccom-
panied by evidence of HIV env reactivity. The
methods demonstrated utilizing both GAG55-ELISA

and ENV9-ELISA shoᴜᴌᴅ ᵗᵒᵛᵉ ⎽ ᵢ routine
blood screening and diɛgnoɔtic a. ⎽.

The ability to identify such ɛɢ-seroposi-
tive and env-seronegative human ᵣ ᵢ samples is
of considerable import ᵢᴄᴄ. F ⁻ᴵⁱ⁻ several
studies, including that ᵢᵢ Aᵢ ᕮ ᵊsented
at the National Institutes ᴄ. Fᵢ ᵗᵘs De-
velopment Conference ᴄᵢᵢ ᵎ ᵢf ᴿoutlne
HTLV-III Antibody Tesᵗ ⎽ ᵎᵎ ul ᵣᵎᵎ ᵎ" (July
7-9, 1986), have indiᴄᵃᵗᵉᴄ tha ᵢ ⎽ᵗᵉd in-
dividuals who initially dᵎsplaᵧ im ᵎᵎ ᵣ reactivity
only to gag specific bᵣᵎᴅs ᵣᵃᵥ ᵣᵤᵉᵣtly dis-
play definite HIV serc ᵒᵊ ᵎᵎ⁻ ᵈᵎᵣᵢᴄᵉd by
env reactivity. It is also ᵎᵎ ᵎᵢ ᵎᵛ gag-
reactive, env-nonreactive sᵊᵣᵎ ᵣᵘre to
other human retrovirusᵊᵎ ᵣuᴄᵎ ᵎᵢ ᵎTLV-II
or HIV-II, and possibly ᵎᵎ ᵎᴄᵒ ᵢᵢ ᵎ ᵎ retro-
viruses. The significanᵎᵉ tᵢ the ᵎ ᵎ ᵎ ᵣansfu-
sion recipient of such HIV ᵣᵃᴄᵎ ᵎᵢ env-non-
reactive sera remains tᵎᵎ be ᵢ ᵉte ᵃᵣd further
studies are essential. ᵢᵎ ᵣ ᵎᵣᵊᵎ ᵣᵤᵣᵊ, blood
from donors with thesᵊᵎ ᵎᵣ ᵎ ᵎᵢ ᵎhould
not be transfused. Thiᵎ ᵎᵪᵎᵢ ᵑᵣ ᵒth the
importance of methods ᵒᵣ ᵎᵉ ᵎᵎ ᵎV gag
proteins and the utility ᵎ ᵎ ᵎ ᵢᴳᵎ ᵎᵣose.

<p align="center">EXᵢ ᵎᵢPᴌ ᵤ</p>

Analysis of Human Antᵎ ᵎ ᵎᵎ ᵎ ᵎᵢ coli-
Expressed env- and gaᵤ ⎽ ᵎ ᵎᵣᵎ

Although the HIV ᵎᴸᵎᴄᴬᵎ ᵎ ᵒᵛᵉᵣᵎᵤᵎ and
sensitive tool for screᴄ ᵎᵣᵎ ᵎf ᵣ ⎽ for the
presence of HIV-speci. ᵎ ᵎᵧ uti-
lizing total virus antigeᵢ ⎽ ᵎ orma-
tion concerning which ᵎᵒᵣ ᵎ ᵎnents
of the virus are immuᵢ ᵊᵢ ᵎ sera.
Immunoblot or immuᵣᵎ ⎽ ᵎ ⎽s can
be used to identify ᵎ ᵎᵎ ⎽ᵎᴄ⎽ ᵎᵧ to ᵎecific
viral components. To ᵎⁱ⁻ᵎ ᵗʰᵊ ᵎᵗion ᵎᵎ HIV-
seropositivity is base ᵎ ᵎ ᵎᵢ ᵒt ᵣᵊᵎctivity
with both gag- and env ᵊᵣ ᵎ ᵎᵢ.

As an alternative ᵎ ᵎ ᵎ ᵎ im-
munoprecipitation metᵎ ᴊᵎ ᵎ rela-
tive levels of antibodᵎᵎ ᵣ ᵎ and
env proteins, we havᵎ ᵎ env-
specific ELISAs, usin ᵎ ⎽ᵎsed ᵎAG55
and ENV9 as antigensᵎ ᵎᵎᵎᵛ⁹ ᴄᵎ ᵎ amiᵣᵎ ᵎ acids
corresponding to the ⎽ ᵎᵣᵣ ᵎs of ᵎᵎᵉ C-
terminus of the HIVᴮᴴ₁ᵢ ᵎᵑ p1 ᵣᵃᵢᵣᵎ ᵎᵢd 240
amino acids of the N-ᵗ ᵎᵎ main.
Methods for detection ᵎ rela-
tive levels of antibodiᵎ ᵎ ᵎᵎ an-
tigenic components oᵎ ᵎ ᴊ env-

encoded proteins, provides information that may be
useful for the diagnosis and prognosis of HIV-
infected individuals. The ELISA format provides
considerable advantages compared to immunoblot
and immunoprecipitation methods. The ELISA pro-
cedure is sensitive, quantitative, rapid, non-radioac-
tive, and relatively inexpensive and simple to run.

Recently several ELISA assays have been de-
veloped which utilize as antigen proteins expressed
in E. coli to detect antibodies to the AIDS virus
[Dowbenko, et al., Proc. Natl. Acad. Sci. 82: 7748-
7752 (1985); Stelmer, et al., Virology 150:283-290
(1986); Cabradilla et al., Biotechnology 4: 128-133
(1986); Chang et al., Biotechnology 3:905-909
(1985); Shoeman et al., Anal. Biochem. 161:370-
379 (1987)]. Previous recombinant gag-derived
ELISAs which have been described only contain
subregions of gag, such as p24^{gag}, and do not
contain sequences corresponding to the entire gag
coding sequence. Since the GAG55 represents the
complete gag coding sequence, the GAG55-ELISA
may detect HIV gag-specific antibodies in human
sera that are not detected by ELISAs employing
subregions of gag. In particular, GAG55 is pre-
ferred to other reported recombinant gag antigens
because the complete p17^{gag} region is represent-
ed. In addition, GAG55 and GAG17 do not contain
any non-HIV or non-HIV gag amino acid se-
quences. These antigens are preferred for antibody
detection because of the specificity for gag and of
the reduced probability of detecting false-positive,
non-HIV immunoreactivity.

The HIV gag-specific and env-specific antibody
levels were quantitated in human sera from in-
dividuals of known clinical status, using both the
GAG55-ELISA and ENV9-ELISA. Sera categorized
as being from AIDS patients, HIV-seropositive (non-
AIDS), and HIV-seronegative individuals were ob-
tained from Duke University. These sera were ana-
lyzed using the GAG55 and ENV9 ELISAs. The
results of these assays are shown in Figure 7.

As shown in Figure 7, the gag-specific appar-
ent antibody levels in many of the AIDS patient
sera are reduced compared to the gag-specific
apparent antibody levels in non-AIDS, HIV-
seropositive sera. This effect is selective, since the
HIV env-specific antibody levels do not distinguish
the AIDS and non-AIDS seropositive groups. The
GAG55-ELISA alone is obviously limited as an HIV
screening assay since many of the characterized
HIV positive sera were not detected as positive
(see also Example 6, Figure 6). This does not,
however, preclude the use of a gag-specific ELISA
as an important component of diagnostic assays.
For example, many sera exhibit HIV gag im-
munoreactivity without detectable env-specific im-
munoreactivity (Example 6). Moreover, quantitation
of the relative antibody levels specific to gag and

env and other HIV antigenic compc ... may be useful for monitoring HIV-as o iated disease. The results presented indicate tha; it may be possible to develop a correlation bet een the serum reactivity on the GAG55 and ENV. 'SA ssays and the stage of disease. The pre .. da'a pr vide evidence that quantitation by E ! A o. .he r ative HIV env-specific and gag-specifi ntibc Ic Is in human sera is useful for both .craenir ar diagnostic analysis of human sera.

Claims

1. A recombinant plasm ' ap e o' igh lev l expression in E. coli of a nfus ' p ide ccr- responding to the complet ; 'V (` ing sa- quence and exhibiting the anti enic..y ... the com- plete HIV p55ᵍᵃᵍ protein, cc....sting essentially of plasmid pBR322 and DNA coding for amino acids 1 to 512 of HIV protein p! ' ', with a translation start signal and an ATG/Me :is ation start codon downstream from a prom c ted rom t' e group consisting of the E. · lac e c ron pr - moter, the E. coli tryptopha ro ru 'er, a d the phage lambda P_L promc r

2. A recombinant plas: ' Clain 1 having incorporated therein a trans' .n s'art signai which is an HIV sequence imm " 'ey 5´ of the c⁻g ATG/Met translation s . :odon or an oligonucleotide linker as de , i F am 2 1.

3. A recombinant plas. , . m vher n the gag ATG/Met translatic . ·r . part of an Ncol restriction endonuc . c

4. An E. coli cell transf ' v .h ; n'asmic of anyone of claims 1 to 3.

5. An HIV gag-encodec n de expressed by a cell of Claim 4.

6. A recombinant plasn . at'le c' igh level expression in E. coli of é .. r e c - responding to the compl ⁻ p1 9 coding sequence and exh.. : , .icity of the complete HIV p17ᵍᵃᵍ p :' r s ' ess n- tially of plasmid pBR322 er.. .A cod...j for a i- no acids 1 to 132 of HIV .' e p5 5 wi.. a translation start signal and .G/Met .ransla. .n start codon downstream fr remo' select d from the group consistin : 'act e operon promoter, the E. t- 2 n promoter, and the phage l: .

7. A recombinant pla : ' ha' ng incorporated therein a tra' 1 : .r , ' h is an HIV sequence imm⁻ ' 5´ 1e g ATG/Met translation : :o:o' r n oligonucleotide linker as d⁻ F a⁻ l⁻ 1.

8. A recombinant pla: . he 'n the gag ATG/Met translati te ° : .)f an Ncol restriction endonu : e

9. An E. coli cell transformed with a plasmid of anyone of claims 6 to 8.

10. An HIV gag-encoded peptide expressed by a cell of Claim 9.

11. An E. coli-expressed recombinant nonfused peptide corresponding to the complete HIV p55ᵍᵃᵍ coding sequence and exhibiting the antigenicity of the complete HIV p55 ᵍᵃᵍ protein.

12. An E. coli-expressed recombinant nonfused peptide according to Claim 11 which is encoded by nucleotides numbered 334 to 1869 of the HIV genome, as shown in Figure 1.

13. An E. coli-expressed recombinant nonfused peptide corresponding to the complete p17 sub-region of the HIV p55ᵍᵃᵍ coding sequence, and exhibiting the antigenicity of the complete HIV p17ᵍᵃᵍ protein.

14. An E. coli-expressed recombinant nonfused peptide according to Claim 13 which is encoded by nucleotides numbered 334 to 729 of the HIV genome, as shown in Figure 1.

15. In a diagnostic kit used for detecting anti- bodies to HIV in a biological sample wherein the sample is contacted with a peptide and im- munoreactivity is detected, the improvement com- prising employing a peptide of anyone of claims 5, 10, 11, and 13.

16. In a process for detecting antibodies to HIV in a biological sample, comprising contacting said sample with a peptide which is immunoreactive with said antibodies and detecting immunoreac- tivity, the improvement comprising employing a peptide of anyone of claims 5, 10, 11, and 13.

17. A vaccine protective against HIV compris- ing an effective protective amount of a peptide of anyone of claims 5, 10, 11, and 13 in a pharmaceu- tically acceptable carrier.

F I G. IA

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                        228                                           255
GAA AGC GGA A . (      .H  AGC TCT CTC GAC GCA GGA CTC GGC TTG CTG AAG
Glu Ser Gly L,. L.     ..  Ser Ser Leu Asp Ala Gly Leu Gly Leu Leu Lys


                        282                                           309
CGC GCA CGG C.,  .G ...  GG GGC GGC GAC TGG TGA GTA CGC CAA AAA TTT TGA
Arg Ala Arg C1r. L       -.. Gly Gly Asp Trp  .  Val Arg Gln Lys Phe  .


                        336                                           363
CTA GCG GAG C . /     AG  GAG ATG GGT GCG AGA GCG TCA GTA TTA AGC GGG
Leu Ala Glu A... ..   Or. Glu MET Gly Ala Arg Ala Ser Val Leu Ser Gly


                        390                                           417
GGA GAA TTA C . .      .. A AAA ATT CGG TTA AGG CCA GGG GGA AAG AAA AAA
Gly Glu Leu A. . .       Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys


                        444                                           471
TAT AAA TTA A. .      GT. TGG GCA AGC AGG GAG CTA GAA CGA TTC GCA GTT
Tyr Lys Leu L,. ..    .. Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val


                        498                                           525
AAT CCT GGC C.. .      . TCA GAA GGC TGT AGA CAA ATA CTG GGA CAG CTA
Asn Pro Gly L.. .        Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu


                        552                                           579
CAA CCA TCC C .       .G TCA GAA GAA CTT AGA TCA TTA TAT AAT ACA GTA
Gln Pro Ser L.. ..    .., Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val


                        606                                           633
GCA ACC CTC T..        . CAA AGG ATA GAG ATA AAA GAC ACC AAG GAA GCT
Ala Thr Leu .., .        Gln Arg Ile Glu Ile Lys Asp Thr Lys Glu Ala


                        660                                           687
TTA GAC AAG A.         .. CAA AAC AAA AGT AAG AAA AAA GCA CAG CAA GCA
Leu Asp Lys ... .        .. Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala


                        714                                           741
GCA GCT GAC A.. .      . AGT CAG GTC AGC CAA AAT TAC CCT ATA GTG CAG
Ala Ala Asp ...  .       Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln


                        768                                           795
AAC ATC CAG C          .. CAT CAG GCC ATA TCA CCT AGA ACT TTA AAT GCA
Asn Ile Gln ... ..       .. His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala


                        822                                           849
TGG GTA AAA C          AAG GCT TTC AGC CCA GAA GTA ATA CCC ATG TTT
Trp Val Lys ... .        Lys Ala Phe Ser Pro Glu Val Ile Pro MET Phe
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FIG. 1B

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                              876                                        903
·TCA GCA TTA TCA GAA GGA ... ACC CCA CAA GAT TTA AAC ACC ATG CTA AAC ACA
 Ser Ala Leu Ser Glu Gly ... Thr Pro Gln Asp Leu Asn Thr MET Leu Asn Thr


                              930                                        957
 GTG GGG GGA CAT ... GCA G.C ATG CAA ATG TTA AAA GAG ACC ATC AAT GAG GAA
 Val Gly Gly His Gl. Ala Ala MET Gln MET Leu Lys Glu Thr Ile Asn Glu Glu


                              984                                       1011
 GCT GCA GAA TGG GAT AGA CTA CAT CCA GTG CAT GCA GGG CCT ATT GCA CCA GGC
 Ala Ala Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly


                             1038                                       1065
 CAG ATG AGA GAA C.. AGG G A AGT GAC ATA GCA GGA ACT ACT AGT ACC CTT CAG
 Gln MET Arg Glu Pr. Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln


                             1092                                       1119
 GAA CAA ATA GCA T.. ATG ACA AAT AAT CCA CCT ATC CCA GTA GGA GAA ATT TAT
 Glu Gln Ile Gly Trp MET Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr


                             1146                                       1173
 AAA AGA TGG ATA ... CTG ..A TTA AAT AAA ATA GTA AGA ATG TAT AGC CCT ACC
 Lys Arg Trp Ile ... Leu Gly Leu Asn Lys Ile Val Arg MET Tyr Ser Pro Thr


                             1200                                       1227
 AGC ATT CTG GAC A. AGA CCA GGA CCA AAA GAA CCT TTT AGA GAC TAT GTA GAC
 Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp


                             1254                                       1281
 CGG TTC TAT AAA    CTA A A GCC GAG CAA GCT TCA CAG GAG GTA AAA AAT TGG
 Arg Phe Tyr Lys ... Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp


                             1308                                       1335
 ATG ACA GAA A... .. TTG C.C CAA AAT GCG AAC CCA GAT TGT AAG ACT ATT TTA
 MET Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu


                             1362                                       1389
 AAA GCA TTG GGA ... .CG GCT ACA CTA GAA GAA ATG ATG ACA GCA TGT CAG GGA
 Lys Ala Leu Gly P.. Ala Ala Thr Leu Glu Glu MET MET Thr Ala Cys Gln Gly


                             1416                                       1443
 GTA GGA GGA CC. G . CAT A.G GCA AGA GTT TTG GCT GAA GCA ATG AGC CAA GTA
 Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala MET Ser Gln Val


                             1470                                       1497
 ACA AAT ACA G.    ATA ATG ATG CAG AGA GGC AAT TTT AGG AAC CAA AGA AAG
 Thr Asn Thr Ala ... Ile MET MET Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys


                             1524                                       1551
 ATG GTT AAG T.. T.. AAT TCT GGC AAA GAA GGG CAC ACA GCC AGA AAT TGC AGG
 MET Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Thr Ala Arg Asn Cys Arg
```

24

F I G. IC

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                                      1578                                      1605
GCC CCT AGG AAA AAG GGC TGT TGG AAA TGT GGA AAG GAA GGA CAC CAA ATG AAA
Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln MET Lys


                                      1632                                      1659
GAT TGT ACT GAG AGA CAG GCT AAT TTT TTA GGG AAG ATC TGG CCT TCC TAC AAG
Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser Tyr Lys


                                      1686                                      1713
GGA AGG CCA GGG AAT TTT CTT CAG AGC AGA CCA GAG CCA ACA GCC CCA CCA TTT
Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Phe


                                      1740                                      1767
CTT CAG AGC AGA CCA GAG CCA ACA GCC CCA CCA GAA GAG AGC TTC AGG TCT GGG
Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Ser Gly


                                      1794                                      1821
GTA GAG ACA ACA ACT CCC CCT CAG AAG CAG GAG CCG ATA GAC AAG GAA CTG TAT
Val Glu Thr Thr Thr Pro Pro Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr


                                      1848                                      1875
CCT TTA ACT TCC CTC AGA TCA CTC TTT GGC AAC GAC CCC TCG TCA CAA TAA AGA
Pro Leu Thr Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln  .  Arg


                                      1902                                      1929
TAG GGG GGC AAC TAA AGG AAG CTC TAT TAG ATA CAG GAG CAG ATG ATA CAG TAT
 .  Gly Gly Asn  .  Arg Lys Leu Tyr  .  Ile Gln Glu Gln MET Ile Gln Tyr


                                      1956                                      1983
TAG AAG AAA TGA CT  TGC CAG GAA GAT GGA AAC CAA AAA TGA TAG GGG GAA TTG
 .  Lys Lys  .  V  Lys Gln Glu Asp Gly Asn Gln Lys  .   .  Gly Glu Leu


                                      2010
GAG GTT TTA TCA A  TA
Glu Val Leu Ser Lys
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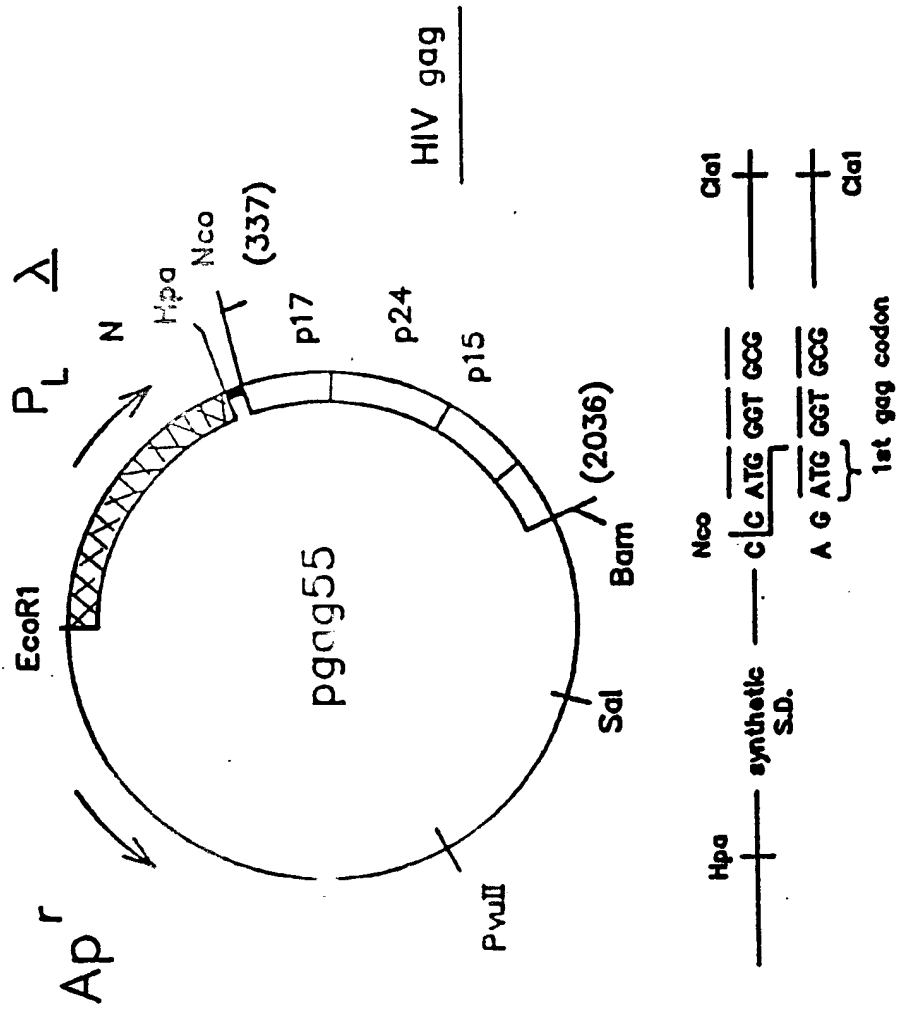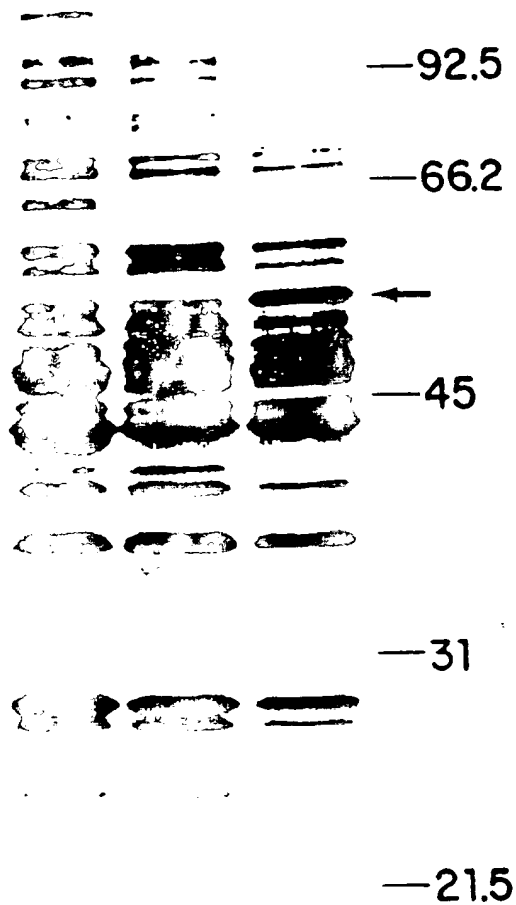
F I G. 2

F I G. 3
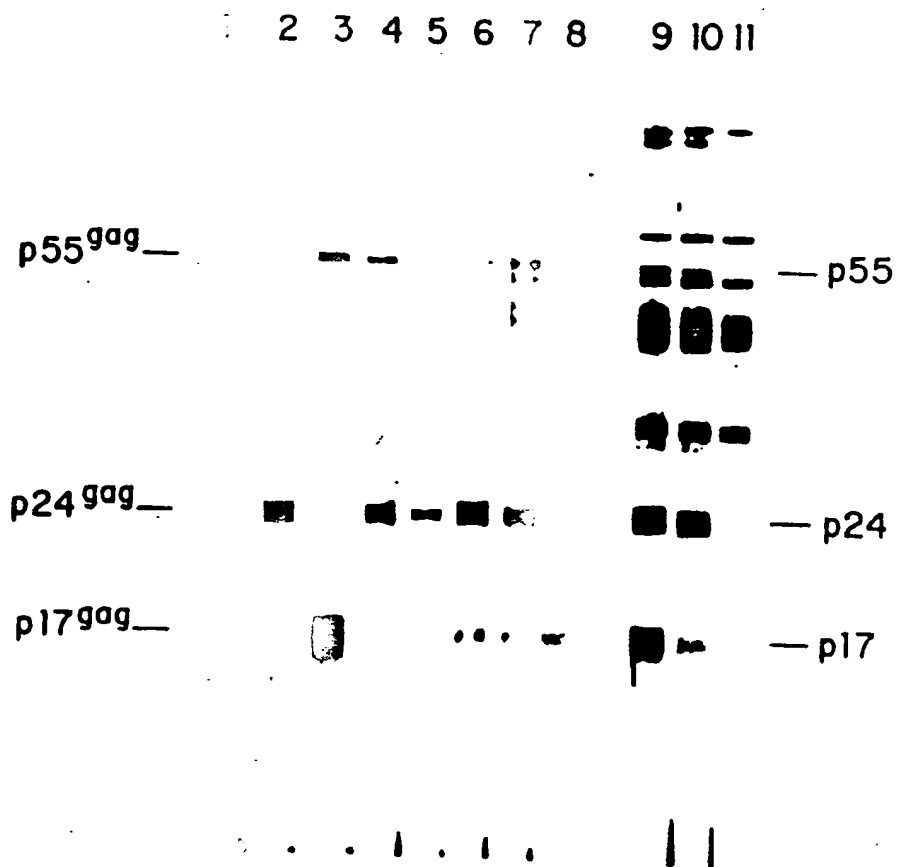
F I G. 4

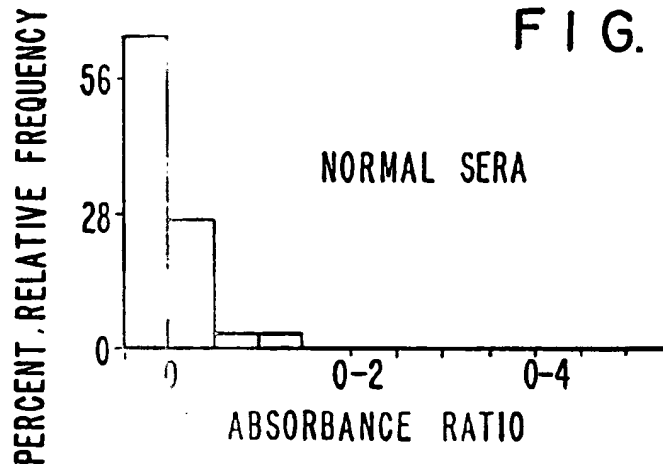1 2 3

—97.4

—68

—43

—25.7

—18.4

F I G. 5

FIG. 6A
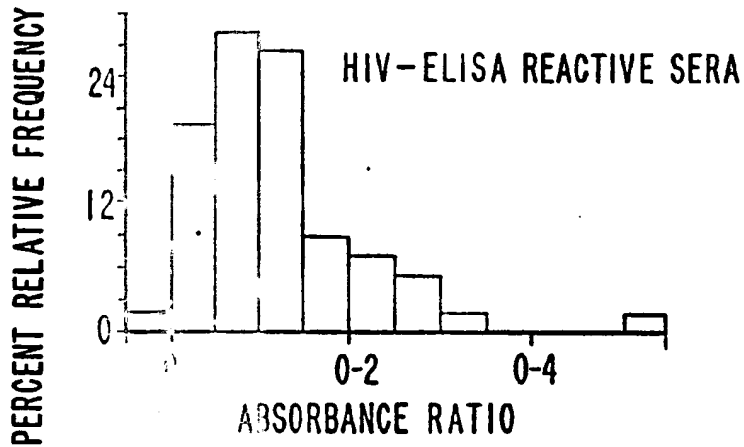
NORMAL SERA

PERCENT, RELATIVE FREQUENCY

ABSORBANCE RATIO

FIG. 6B

HIV-ELISA REACTIVE SERA

PERCENT RELATIVE FREQUENCY

ABSORBANCE RATIO

FIG. 6C

HIV POSITIVE SERA

PERCENT RELATIVE FREQUENCY

ABSORBANCE RATIO

F I G. 7